

Hepatitis B immunity in teenagers vaccinated as infants: an Italian 17-year follow-up study

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Abstract

We assessed the persistence of hepatitis B surface antigen antibody (anti-HBs) and immune memory in a cohort of 571 teenagers vaccinated against hepatitis B as infants, 17 years earlier. Vaccinees were followed-up in 2003 and in 2010 (i.e. 10 years and 17 years after primary vaccination, respectively). When tested in 2003, 199 vaccinees (group A) had anti-HBs <10 mIU/mL and were boosted, 372 (group B) were not boosted because they had anti-HBs ≥10 mIU/mL ($n = 344$) or refused booster ($n = 28$) despite anti-HBs <10 mIU/mL. In 2010, 72.9% (416/571) of participants had anti-HBs ≥10 mIU/mL (67.3% in group A vs. 75.8% in group B; $p = 0.03$). The geometric mean concentrations (GMCs) were similar in both groups. Between 2003 and 2010, anti-HBs concentrations in previously boosted individuals markedly declined with GMC dropping from 486 to 27.7 mIU/mL ($p < 0.001$). Fifteen vaccinees showed a marked increase of antibody, possibly due to natural booster. In 2010, 96 individuals (37 of group A and 59 of group B) with anti-HBs <10 mIU/mL were boosted; all vaccinees of the former group and all but two of the latter had an anamnestic response. Post-booster GMC was higher in group B (895.6 vs. 492.2 mIU/mL; $p = 0.039$). This finding shows that the immune memory for HBsAg persists beyond the time at which anti-HBs disappears, conferring long-term protection.

Keywords: Booster, hepatitis B, immune memory, long-term protection, vaccination

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serious and potentially fatal infection can be prevented by vaccination, and a substantial reduction of new HBV infections, carrier rate and HB-related mortality has been observed in countries where vaccination has been implemented [1–3].

In Italy, universal vaccination of infants and 12-year-old adolescents became mandatory in 1991 [4,5]. At the end of 2003, the first infant cohort vaccinated in 1991 turned 12 years of age. In 2004, the vaccination of 12-year-olds was stopped, whereas vaccination of infants continued. Over 95% vaccination coverage was achieved in a few years with an outstanding record of safety and effectiveness [6]. To date, over 19 million young people under 32 years of age have been vaccinated. Our vaccination campaign has greatly contributed to the decline in incidence of both acute hepatitis B and acute

Introduction

Hepatitis B virus (HBV) is a leading cause of acute and chronic hepatitis, including cirrhosis and hepatocellular carcinoma. This

infection by hepatitis Delta virus (HDV), as a consequence of the biological association between HBV and HDV [7,8].

Although the highly protective efficacy of hepatitis B vaccine has been confirmed, the long-term duration of protection achieved by vaccination remains unclear [9]. Studies on long-term protection induced by hepatitis B vaccination have been mainly conducted in areas of high HBV endemicity. Some studies suggest that vaccine-induced immunity may persist for up to 20 years or longer, after complete primary hepatitis B vaccination [10–14]. Other studies suggest that the immune memory may begin to wane during the second decade after vaccination [15–19]. Moreover, long-term follow-up data are scarce in areas of low endemicity [20–24]. Thus, assessing the persistence of immunity following primary vaccination in infants and adolescents is crucial to determine whether a booster vaccination is needed later in life, when subjects may be at increased risk of HBV infection either due to lifestyle or professional exposure.

In 2003, a large multicentre study was carried out in Italy to assess hepatitis B vaccine-induced immune memory in children who were immunized as infants 10 years earlier. Despite the loss of protective concentrations (≥ 10 mIU/mL) of antibodies to hepatitis B surface antigen (anti-HBs) in a number of vaccinees, a strong immune memory still persisted 10 years after primary immunization, thus suggesting that no routine booster doses of vaccine were necessary for a decade [22]. In 2010, we decided to extend the follow-up of that study by recalling the children, now teenagers, with the aim of assessing the persistence of anti-HBs and immune memory 17 years after primary vaccination.

Materials and Methods

Study participants

A cohort of vaccinees immunized as infants with three paediatric doses of recombinant hepatitis B vaccine (Engerix B, SmithKline Beecham, Biological, Rixensart, Belgium) given at 3, 5 and 11 months of age was first followed-up in 2003 or 10 years after primary vaccination [22], and recalled in 2010 or 17 years after priming. At enrollment, all participants were in good health and none had a history or presented signs or symptoms of clinically overt hepatitis. Exclusion criteria also included congenital or acquired immune disorder, sensitivity or allergy to any component of the study vaccine and having received extra doses of hepatitis B vaccine between 2003 and 2010.

Participants were subdivided into two groups: group A who were given a booster dose of hepatitis B vaccine in 2003 and group B who were not boosted at that time.

Ethics

The study was approved by the Ethics Committee of the University of Milan. Before inclusion, written informed consent was obtained from each participant or, for those still under age (<18 years), from their parents or legal guardians.

Procedures and definitions

Participants were tested for anti-HBs concentrations and the presence of antibody to core antigen (anti-HBc) as a marker of HBV infection. Individuals positive for anti-HBc were tested further for hepatitis B surface antigen (HBsAg) and HBV-DNA, following the same laboratory algorithm applied in the 2003 study [22].

Individuals with anti-HBs concentrations ≥ 10 mIU/mL were considered protected while those with antibody <10 mIU/mL were given a booster adult dose of recombinant hepatitis B vaccine (Engerix B, 20 μ g) and retested 2 weeks later.

An anamnestic response was defined as a four-fold post-booster rise in anti-HBs concentration compared with the pre-booster concentration or an increase up to ≥ 10 mIU/mL for those who had no detectable anti-HBs.

Individuals showing post-booster anti-HBs concentrations <10 mIU/mL were offered two additional vaccine doses at 1 and 6 months after the booster, and retested 1 month after the third dose.

A natural booster (exposure to HBV without infection) was defined as seroconversion to ≥ 10 mIU/mL or at least a four-fold increase in anti-HBs concentration in the sample collected in 2010 versus the sample collected in 2003 in the same subject, without revaccination and no appearance of anti-HBc antibody. To avoid differences due to the use of different kit batches, measurements of antibody concentrations in such samples were repeated in parallel on the same run.

Serological testing

HBsAg, anti-HBc and anti-HBs were detected by commercially available kits (AxSYM HBsAg, CORE and AUSAB, Abbott Park, IL, USA). The measurement range of AxSYM AUSAB was 2–1000 mIU/mL, defined by the detection limit and the maximum of the calibration curve. Samples with anti-HBs ≥ 1000 mIU/mL were diluted with the manual dilution protocol, according to the manufacturer's instructions, to obtain the final sample concentration. Samples below the detection limit (2 mIU/mL) of the assays were recorded as undetectable. HBV-DNA was detected by real-time PCR by the TaqMan HBV test (Roche, Branchburg, NJ, USA) with a 95% detection limit of 6.7 IU/mL (approximately 39 copies per mL).

All samples collected in 2003 were stored at -80°C and thawed in 2010 for this follow-up study.

Statistical analysis

Anti-HBs geometric mean concentrations (GMCs) were compared between participants of group A and group B before and after booster given in 2010 using the non-parametric Mann–Whitney *U*-test. In the case of undetectable anti-HBs antibody concentration, an arbitrary value of 0.5 mIU/mL was assigned to allow for calculation of GMCs. Differences in frequency were tested by the chi-square test or Fisher exact test, when necessary. A *p* value <0.05 was considered to be statistically significant; 95% confidence intervals (95% CI) for GMCs and frequencies were also calculated, when appropriate. Statistical calculations were conducted using Stata Statistical Software, version 11 (Stata Corporation, College Station, TX, USA).

Results

Features of participants

Of the 1212 eligible individuals, 571 (47.1%) were recruited. Of these, 296 (51.8%) were female and 275 (48.2%) were male. The median age at enrollment was 17 (range, 15.5–18.3) years; a median of 16.7 (range, 14.8–18.1) years had elapsed since the last dose of primary vaccination. The enrolled individuals did not significantly differ from those who refused their participation or were untraceable in terms of gender and year of primary vaccination. Of the 571 recruited individuals (Fig. 1), 199 belonged to group A, who were given a booster dose of hepatitis B vaccine in 2003 on account of anti-HBs <10 mIU/mL, and 372 to group B, who were not boosted either because they had antibody ≥ 10 mIU/mL ($n = 344$) or because they refused booster despite antibody concentrations below 10 mIU/mL ($n = 28$).

Serological data at recruitment

In 2010 (Table 1), 416 (72.9%) teenagers had anti-HBs ≥ 10 mIU/mL, while the remaining 155 (27.1%) had antibody that was <10 mIU/mL ($n = 95$) or undetectable ($n = 60$). The proportion of individuals with seroprotective anti-HBs levels was lower in group A than in group B (67.3% vs. 75.8%; $p = 0.03$), while GMC was similar in both groups (27.7 vs. 29.4 mIU/mL; $p = 0.759$). This was despite the fact that in 2003, as a consequence of the booster effect, the GMC was significantly higher in group A than in group B (486.0 vs. 72.1 mIU/mL; $p < 0.001$). Thus between 2003 and 2010, the antibody concentration decay was much higher in group A than in group B (from 486.0 to 27.7 mIU/mL and from 72.1 to 29.4 mIU/mL, or 17.5 vs. 2.5-fold reduction, respectively; $p < 0.001$).

No subject was found to be anti-HBc positive. Fifteen vaccinees of group B, including five with anti-HBs ≥ 10 mIU/mL

and 10 with anti-HBs that was <10 mIU/mL ($n = 7$) or undetectable ($n = 3$) in 2003, showed an antibody seroconversion to over 10 mIU/mL or at least a four-fold increase in anti-HBs concentrations in 2010, probably due to a natural booster response. All these subjects tested negative for anti-HBc, HBsAg and HBV-DNA.

Anamnestic response to booster given in 2010

A total of 96 out of 155 individuals (61.9%) who had anti-HBs concentrations that were <10 mIU/mL ($n = 61$) or undetectable ($n = 35$) at enrollment in 2010 accepted the administration of a booster dose of vaccine. Of these, 37 belonged to the 65 (56.9%) individuals of group A and 59 to the 90 (65.5%) individuals of group B (Fig. 1).

Two weeks after booster (Table 2), all individuals of group A and all but two of group B showed an anamnestic increase in anti-HBs concentration that exceeded the seroprotective threshold, GMC being significantly higher in group B than in group A (895.6 vs. 492.2 mIU/mL; $p = 0.039$). In particular, anti-HBs concentrations rose to over 1000 mIU/mL in 45.8% of individuals in group B versus 32.4% in group A ($p = 0.196$). It is noteworthy that the antibody GMC achieved by all vaccinees (group A plus group B) after booster was higher among those whose pre-booster anti-HBs was <10 mIU/mL but detectable than among those with undetectable pre-booster antibody (1201.6 vs. 285.0 mIU/mL, $p < 0.001$; data not shown).

Among vaccinees who had anti-HBs <10 mIU/mL in 2003, the rate of anamnestic response and GMCs achieved after the booster given in 2010 did not significantly differ between those who had already been boosted in 2003 ($n = 37$) and those ($n = 14$) who had refused the booster (100% vs. 85.7%, $p = 0.073$; 492.2 vs. 214.6 mIU/mL, $p = 0.473$; Table 3).

No adverse events related to the booster dose were reported. Finally, the two vaccinees who did not show an anamnestic response after boosting and received two additional doses of vaccine showed an antibody response 1 month after the third dose of vaccine with concentrations between 100 and 1000 mIU/mL in one case and >1000 mIU/mL in the other (Fig. 1). These two individuals refused booster vaccination in 2003 despite having anti-HBs below 10 mIU/mL and this resulted in antibody being undetectable at testing performed at enrollment in 2010.

Discussion

In 2010, we assessed the persistence of anti-HBs antibody and immune memory in a cohort of 571 teenagers vaccinated against hepatitis B as infants, 17 years earlier. Of these, 199 (group A) had a booster dose of hepatitis B vaccine in 2003,

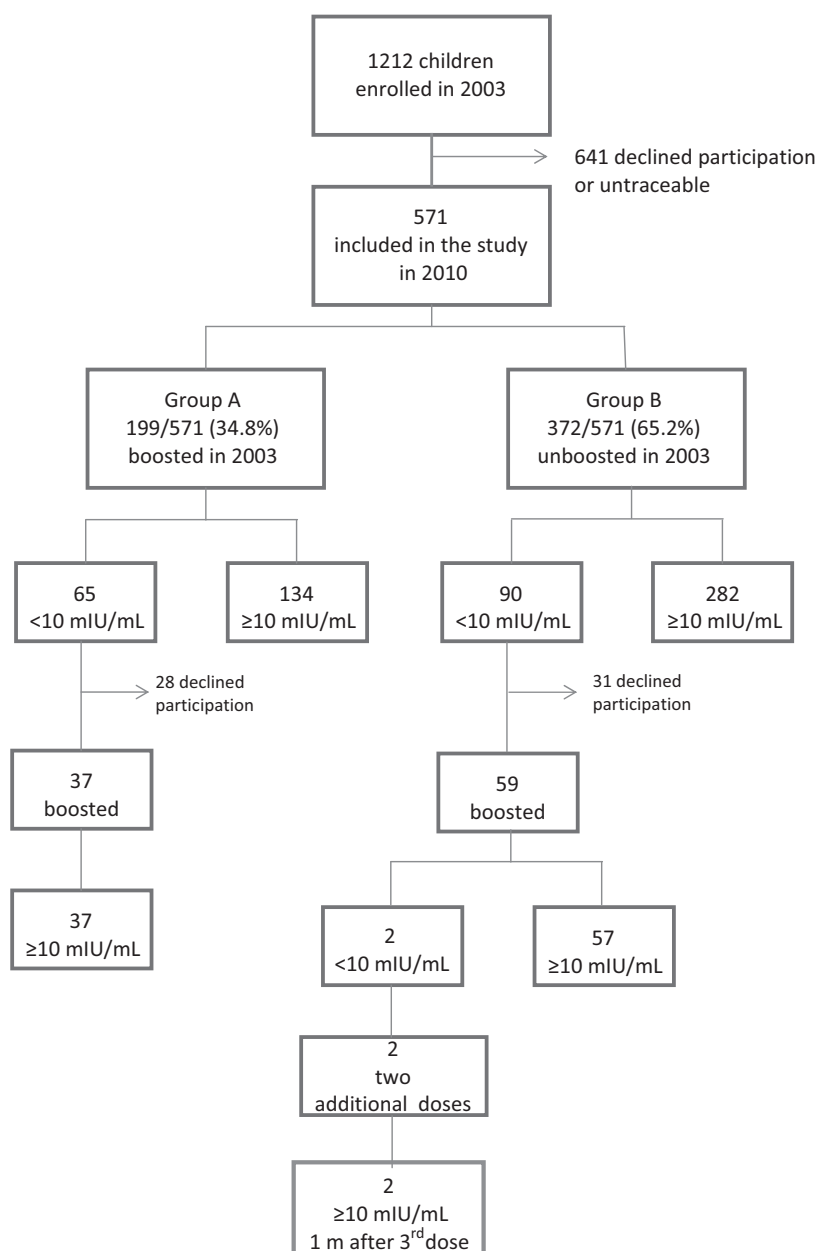


FIG. 1. Study profile.

TABLE 1. Anti-HBs concentrations (mIU/mL) in 571 participants tested in 2010 or 17 years after primary vaccination, according to whether they were boosted (group A) or not (group B) in 2003

Vaccinees	Anti-HBs concentration (mIU/mL)					Subtotal ≥10	GMC (95% CI)
	Undetectable	<10	10–100	>100–1000	>1000		
Group A (n = 199)	36 (18.1) ^e	29 (14.6)	74 (37.2)	45 (22.6)	15 (7.5)	134 (67.3) ^a	27.7 ^b (19.5–39.2)
Group B							
≥10 mIU/mL (n = 344)	8 (2.3)	64 (18.6)	195 (56.7)	71 (20.6)	6 (1.7)	272 (79.1) ^c	34.1 ^d (29.1–40.0)
<10 mIU/mL (n = 28)	16 (57.1)	2 (7.1)	3 (10.7)	6 (21.4)	1 (3.6)	10 (35.7) ^c	4.8 ^d (1.5–15.1)
Subtotal (n = 372)	24 (6.5)	66 (17.7)	198 (53.2)	77 (20.7)	7 (1.9)	282 (75.8) ^a	29.4 ^b (24.7–35.1)
Total (n = 571)	60 (10.5)	95 (16.6)	272 (47.6)	122 (21.4)	22 (3.9)	416 (72.9)	28.8 (24.4–34.0)

^ap 0.03; ^bp 0.759; ^cp <0.001; ^dp <0.001.^eData are numbers (%).

TABLE 2. Anti-HBs concentrations 2 weeks after booster vaccination given in 2010 in group A and group B

Vaccinees	Post-booster anti-HBs concentrations (mIU/mL)						GMC (95% CI)
	Undetectable	<10	10–100	>100–1000	>1000	Subtotal ≥10	
Group A (n = 37)	–	–	4 (10.8) ^c	21 (56.8)	12 (32.4) ^a	37 (100)	492.2 ^b (323.2–749)
Group B (n = 59)	1 (1.7)	1 (1.7)	3 (5.1)	27 (45.8)	27 (45.8) ^a	57 (96.6)	895.6 ^b (531.4–1509.5)
Total (n = 96)	1 (1.0)	1 (1.0)	7 (7.3)	48 (50)	39 (40.6)	94 (97.9)	711.1 (496.6–1018.2)

^ap 0.196; ^bp 0.039.^cData are numbers (%).**TABLE 3.** Proportion of anamnestic response to a booster vaccination given in 2010 in 96 individuals belonging to group A or B according to the anti-HBs concentrations detected at enrollment in 2003.

	Anti-HBs at enrollment in 2003	Post-booster anti-HBs concentration in 2010	
		≥10 mIU/mL	GMC (95% CI)
Group A (n = 37)	<10 mIU/mL	37 (100) ^c	492.2 ^b (323.2–749)
Group B (n = 59)	<10 mIU/mL (n = 14)	12 (85.7) ^a	214.6 ^b (45.8–1005.3)
	≥10 mIU/mL (n = 45)	45 (100) ^a	1396.8 (883.5–2208.3)

^ap 0.073; ^bp 0.473.^cData are numbers (%).

while 372 (group B) were not boosted. Taken together, 72.9% of vaccinees still had protective levels of antibody when tested in 2010. The proportion of vaccinees who maintained anti-HBs levels ≥10 mIU/mL was higher in group B than in group A (75.8% vs. 67.3%). It is noteworthy that GMC showed a greater reduction between 2003 and 2010 in the boosted group than in the unboosted group (17.5 vs. 2.5-fold reduction). This suggests that the kinetics of anti-HBs decay and, consequently, the persistence of antibody over time mainly depend on the magnitude of the peak antibody level achieved after primary immunization rather than on the peak reached following booster administration, but other factors such as genetic factors and body mass index related to the individual may have played a role. A rapid fall in post-booster antibody levels was also reported by an Alaskan study showing that only 41% of vaccinees maintained antibody ≥10 mIU/mL 1 year after booster administration [12]. Similar findings were found in mentally retarded patients as well as in healthcare workers, who experienced a sharp drop in antibody GMC in the first post-booster year [25,26]. Thus, this antibody decline strongly suggests that post-booster anti-HBs concentrations tend to wane rapidly and eventually return to the levels detected before the booster dose.

In this study, regarding the proportion of individuals who maintain protective anti-HBs levels over time, we can infer that the 199 vaccinees who had pre-booster antibody <10 mIU/mL in 2003 would still be below the level of 10 mIU/mL in 2010, if

unboosted. Thus, adding these 199 vaccinees to those (n = 90) who lost antibody between 2003 and 2010, we can assume that 289 out of 571 vaccinees (50.6%) were expected to have antibody concentrations <10 mIU/mL, 17 years after primary vaccination as infants.

Natural booster responses were found in 15 (2.6%) vaccinees. The fact that 10 of these vaccinees, despite having anti-HBs below protective levels (seven with anti-HBs <10 mIU/mL and three with undetectable antibody), showed a strong anamnestic response in the absence of serological signs of HBV breakthrough infections, suggests that cellular immunity lasts much longer than humoral immunity. Similar findings were reported in Alaska and in the Czech Republic, where 8.2% of 1530 vaccinees followed for 10 years after immunization and 6% of vaccinated children born to HBsAg-positive mothers had a natural booster response [26,27]. In Thailand, natural booster responses were observed between 10 and 20 years after the start of primary vaccination of children born to HBsAg carrier mothers [28,29].

Overall, the booster dose of vaccine given in 2010 elicited a strong anamnestic response in 98% of vaccinees. The increase in anti-HBs concentrations was higher in those with pre-boosting anti-HBs <10 mIU/mL than in those with undetectable antibody before boosting. This finding indicates that although anti-HBs concentrations decrease over time to levels of antibody that are <10 mIU/mL or even undetectable, a strong immune memory still persists. This is consistent with the outcome of long-term studies carried out in high endemicity countries [9–13], although very recent studies [14–18] show increased failure to respond to a booster dose of vaccine, two decades after primary vaccination. Surveillance and additional long-term follow-up of vaccinated cohorts are needed to clarify this issue.

Finally, two vaccinees who did not show an anamnestic response after boosting developed protective antibody levels after an additional full course of vaccination. The fact that both of these vaccinees had anti-HBs <10 mIU/mL in 2003, which became undetectable in 2010, suggests that they were likely to be hyporesponsive to the primary vaccination and that the ability to respond to a booster might wane over time,

especially in individuals with low post-priming antibody levels. However, the small number of vaccinees examined with this profile does not allow definitive conclusions.

A limitation of this study is that the administration of a booster dose to those with anti-HBs <10 mIU/mL 10 years after vaccination may have somehow overestimated the persistence of vaccine-induced immunity at 17 years after primary vaccination.

Despite this, our study clearly shows that the immune memory for HBsAg persists beyond the time at which anti-HBs disappears, conferring long-term protection.

A concentration of anti-HBs ≥ 10 mIU/mL measured after a primary course of vaccination is universally considered protective, but it is questionable whether this threshold can be periodically used for the evaluation of long-term protection, because the drop in antibody below this value does not necessarily mean susceptibility to HBV infection. Actually, most vaccinees with such a serological profile show a rapid and vigorous anamnestic response when boosted.

In conclusion, our finding indicates that the immune memory for HBsAg achieved by primary immunization with three paediatric doses of recombinant hepatitis B vaccine given in the first year of life lasts for at least 17 years and additional booster doses are not needed at this time to maintain long-term protection. However, surveillance of vaccinated cohorts should continue.

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Transparency Declaration

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participation in a Scientific Advisory Meeting on Pertussis. All other authors declare that they have no conflict of interest.

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Appendix I: Study Group

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